47. (Amended) The maize plant, or parts thereof, of claim 21, further comprising one or more single gene conversions.

48. (Amended) The maize plant, or parts thereof, wherein the one or more single gene conversions of claim 47 comprise a dominant allele.

49. (Amended) The maize plant, or parts thereof, wherein the one or more single gene conversions of claim 47 comprise a recessive allele.

Please add new claims 50 and 51

50. (New) The seed of claim 1 wherein said seed further comprises genetic or cytoplasmic male sterility.

51. (New) The maize plant of claim 21, wherein said plant further comprises genetic or cytoplasmic male sterility.

REMARKS

Claims 1-51 are now pending in the application. Claims 3, 5, 14, 15, 16, 19, 20, 22, 24, 33, 34, 35, 37, 41, 43, 45, 46, 47, 48, and 49 have been amended. New claims 50-51 have been added. Support for the amendments and new claims 50-51 can be found in the specification. In particular for the new claims 50 and 51, support for the limitation of "said plant further comprises genetic or cytoplasmic male sterility" can be found starting on line 34 of page 1 and continuing through line 14 on page 3. No new matter has been added by amendment or addition of new claims. Reexamination and reconsideration of the claims as amended are respectfully requested.

CLAIM OBJECTIONS

Examiner objects to claims 1, 6, 21, 25, 37, and 40 for the inclusion of a blank line where the ATCC accession number should be included. Applicant respectfully submits that the pertinent claims will be amended at such time the actual deposit has been made as set forth in 37 CFR §§ 1.801-1.809. Once notice of allowable claims has

been received by Applicant, a deposit will be made with the ATCC and the claims will be amended to recite the accession number.

With regard to the deposit of inbred line PH48V, Applicant wishes to note that:

(a) during the pendency of this application access to the invention will be afforded to the Commissioner upon request:

(b) all restrictions upon availability to the public will be irrevocably removed upon granting of the patent;

(c) the deposit will be maintained in a public depository for a period of thirty years, or five years after the last request for the enforceable life of the patent, whichever is longer;

(d) a test of viability of the biological material at the time of deposit will be conducted (see 37 C.F.R. § 1.807); and

(e) the deposit will be replaced if it should ever become inviable.

Applicant wishes to state that the actual ATCC deposit will be delayed until the receipt of notice of otherwise allowable subject matter. Once such notice is received, an ATCC deposit will be made, and the claims will be amended to recite the ATCC deposit number. In addition, Applicant submits that at least 2,500 seeds of PH48V will be deposited with the ATCC.

Examiner points out an error in claim 37 where a comma should be inserted after "PH48V" and an error in claim 47 where the word "plants" should be replaced with the word "plant". Applicant has amended claims 37 and 47 to reflect the suggestions made by the Examiner.

REJECTIONS UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Examiner rejects claims 3 and 22 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Examiner states that the recitation of "wherein said plant is male sterile" is indefinite because the claims from which they depend are drawn to plants which are not male sterile. Examiner suggests that if the "Applicant intended to claim a plant derived from the plant of the preceding claim, further comprising a genetic factor for male sterility, then the claims should be so amended."

The Applicant has amended claims 3 and 22 and added claims 50 and 51, thus obviating this ground of rejection. The Applicant submits that though the deposited line is not male sterile, claims 3, 22, 50 and 51 are submitted as part of the invention

because they constitute a variation of the invention which is well known to one skilled in the art of plant breeding and seed production. Inbreds are routinely made male sterile by physically removing the tassel from the plant or by routine plant breeding methods of moving nuclear genes or cytoplasmic genes from one cultivar to another (Poehlman et al. , 1995, page 332 (A9), also see line 34 of page 1 through line 14 of page 3 of specification).

Examiner rejects claims 5, and 24 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Examiner states "claims are indefinite in their recitation of 'the... protoplasts' which lacks antecedent basis in the preceding claims. The claims should be amended to delete 'the' before 'cells' and to insert --of the tissue culture-- after 'protoplasts'." Claims 5 and 24 have been so amended and Applicant thanks the Examiner for his suggestion.

The Examiner rejects claims 14, 33, 41, 45, and 46 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner states that the recitation of "high", "strong", "above average", and "tall" is unduly narrative and does not clearly set forth the claimed characteristics or degree of their expression. Applicant has amended claims 14, 33, 41, 45, and 46 thus obviating this ground for rejection.

Applicant has amended the claims using the term "not significantly different from PH48V when determined at a 5% significance level..." as a definitive term. In the specification pages 35-49, the tables and the written descriptions of the tables show mean trait values, including Southern Leaf Blight resistance, Northern Leaf Blight resistance, Gray Leaf Spot resistance, yield, stalk lodging resistance, root lodging resistance, staygreen, plant height, and ear height, as being significantly different when tested at the 5% significance level. The standards against which the listed traits should be compared are the mean values for those traits exhibited by PH48V or a PH48V-derived line in a side-by-side comparison or other similar environmental conditions. For example, on page 35 lines 1-21 of the specification it discusses that PH48V demonstrates significantly taller plant height, significantly higher ear placement, significantly higher staygreen scores, and significantly better staygreen scores when compared to other individual inbreds. The discussions of Tables 4A-4E on pages 35-37 discuss significant differences in yield, staygreen scores, ear height, resistance to stalk lodging, Gray Leaf Spot resistance and Southern Leaf Blight when comparing PH48V hybrids and other hybrids. The Applicant would also like to point out that one of ordinary

skill in the art of plant breeding would know how to evaluate the traits of two inbred maize lines to determine if they are not significantly different to a 5% significance level in the expression of a given trait. On pages 275-276 in Principles of Cultivar Development (1987) Fehr writes "Two or more independent comparisons of lines in a test provide a means of estimating whether variation in performance among lines is due to differences in genetic potential or to environmental variation." A copy of Fehr, pages 261-286, is attached to this Amendment and Request for Reconsideration as Appendix A. As was done by the Applicant in the specification, mean trait values would be used to determine whether the trait differences are significant. Further, the claims, as amended, require that the traits be measured on plants grown in the same environmental conditions. Given the amendment to the claims and supporting discussion, Applicant submits that the claims are definite and requests that the Examiner reconsiders and withdraws the rejection.

Examiner rejects claims 15 and 34 under 35 U.S.C. §112, second paragraph, as being indefinite. The Examiner writes, "Claims 15 and 34 are indefinite in their recitation of 'include', as it is unclear whether this is an open or closed term. Furthermore, the claims are indefinite for failing to positively recite essential method steps, in their recitation of 'obtaining' in the body of the claim." Claims 15 and 34 have been amended to recite that in addition to obtaining plants, the method comprises "employing said plant or parts as a source of breeding material using plant breeding techniques." Various breeding techniques for employing said plant or plant parts as a source of breeding material are well known in the art and many of such techniques are described on pages 3-4 of the specification. These amendments obviate the grounds for rejection.

Examiner rejects claims 16 and 35 as being "indefinite in their recitation of 'The maize breeding program of claim 15 [or 34]', since the preceding claims were drawn to a method rather than a breeding program." Applicant thanks the Examiner for pointing this out and claims 16 and 35 have been amended to refer to "method" of the previous claims.

Examiner rejects claims 19-20 and 48-49 as being "indefinite in their recitation of 'The single gene conversion(s) of claim 18 [or 47]' since the preceding claims were drawn to maize plants rather than single gene conversions." Applicant has amended claims19-20 and 48-49 to refer the maize plants of the previous claims. For example claim 19 now reads, "The maize plant, or parts thereof, wherein the one or more single gene conversions of claim 18 comprise a dominant allele." Applicant submits that this rejection has now been overcome.

Examiner rejects claims 37-39 as being indefinite in their recitation of steps (c) and (e). The Examiner states, "The process step in part (c) of 'identifying said inbred plants' is both vague and indefinite in failing to set the metes and bounds of the invention. It is noted that a hybrid plant comprises at least two parents and, in the claimed invention, must comprise at least PH48V as one parent. The method steps do not teach or identify how the person having skill in the art would identify PH48V from the other parent(s) of the hybrid and as such fail to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Furthermore, the steps of identifying the plants with decreased vigor and identifying plants with homozygous genotypes fails to distinguish the parent, which is not PH48V. Additionally noted is the limitation in part (e) 'in a manner which preserves the homozygosity of said inbred...' which fails to set the metes and bounds of the claimed invention, or to provide a positive method step to meet the requirement to preserve the homozygosity of the plant. Amending claim 37 to recite a positive method step, such as selfing to preserve homozygosity or further limitation to the degree of homozygosity, would serve to obviate this portion of the rejection."

Applicant respectfully traverses this rejection. Claim 37 and dependent claims 38 and 39 are clearly directed to a process for producing inbred line PH48V whereby the starting material is an inbred found in a bag of hybrid seed wherein PH48V is one of the parents of said hybrid. The present application clearly teaches at page 5, line 21, to page 6, line 10, that the identification of a selfed parent, usually the female parent, is a routine practice to one of ordinary skill in the art. The self-pollinated inbreds found in hybrid seed are usually the result of incomplete removal or incomplete inactivation of the pollen from the female parent in the production of hybrid seed. This inadvertently self-pollinated seed may then be unintentionally harvested and packaged with the hybrid seed.

As the male rows in a production field are routinely harvested separately or culled from those female rows producing the hybrid seed, one of skill in the art would realize that inadvertent selfs in a bag of hybrid seed are most likely those of a female parent. The present application teaches that the self-pollinated plants can be identified and selected due to their decreased vigor. In addition, analysis can be conducted utilizing molecular marker techniques known to those of ordinary skill in the art whereby lines suspected to be inbred parents can be shown to have predominantly homozygous genetic composition.

Claim 37 is clearly directed to the parent that is PH48V. Inbred line PH48V has been clearly described through both written description and examples (see Table 1 on

pages 17-19 and Tables 2A, 2B and 2C on pages 38-41). However in an effort to expedite prosecution, Applicant has amended claim 37 to recite that morphological and/or physiological data is collected in order to identify said inbred parent as PH48V. As such the claim now requires that data be collected to identify the parent as PH48V. Earlier Applicant recited in step (c) that the PH48V parent plant was identified simply after planting the seed of a hybrid, one of whose parents is inbred PH48V. The claim now recites that one must plant the hybrid, grow the plants, identify parent plants, select the parent plants, control pollination by self-pollinating to preserve homozygosity of the parent plant, and also collect sufficient data to identify the parent as PH48V. It is submitted that this will allow for the identification of PH48V to distinguish it from the other parent even if the other parent is closely related to PH48V.

In light of the above remarks, Applicant submits that claim 37, and therefore dependent claims 38 and 39, now clearly distinguish that the inbred line to be selected is PH48V and not the second parent.

Examiner rejects claim 43 as being "indefinite in its recitation of 'further derived maize plant' as it is unclear whether additional breeding steps besides those of claim 42 were included in the derivation of the 'further derived' plant." Applicant has amended claim 43 thus obviating the grounds for rejection. The amended claim 43 now clearly claims "The further PH48V-derived maize plant or parts thereof, produced by the method of claim 42."

REJECTIONS UNDER 35 U.S.C. §§ 102 and 103

The Examiner rejects claims 14, 17, 33, 36, and 46 under 35 U.S.C. § 102(b) as anticipated by or, in the alternative, under 35 U.S.C. § 103 (a) as obvious over Arthur (U.S. Patent 5,723,739). The Examiner states, "Arthur teaches a maize inbred with high resistance to Northern Leaf Blight, high yield, and strong staygreen (see e.g., column 6, lines 7-14; column 7, lines 1-6). The maize plant taught by Arthur differs from the claimed maize plants only in their derivation from PH48V. However, the mere inclusion of PH48V in the pedigree of the claimed plants would not distinguish them from the prior art plants, particularly since the number of other parents, crosses or generations is not specified in the claims, wherein increasing the number of each parameter would result in a decrease in PH48V-derived genes. See *In re Thorpe*, 227 USPQ 964, 966 (Fed. Cir. 1985), which teaches that a product-by-process claim may be

properly rejectable over prior art teaching the same product produced by a different process, if the process of making the product fails to distinguish the two products."

The Applicant respectfully traverses this rejection. Applicant submits that though PH48V and LH281 exhibit some traits in common what is being claimed is not the trait but the unique combinations of alleles contained in PH48V which result in a given trait. These unique combinations of genetics and traits in PH48V are what will give rise to the claimed plants resulting from breeding with this material.

Applicant submits that when looking at inbred maize lines it is possible to find traits that are similar between the two lines. However, to say that there are similarities in phenotype between two varieties is not the same as saying that the two lines have the same morphological and physiological characteristics as a whole, or that one is an obvious variant of the other. Further, similarity in phenotype does not mean that the two varieties have the same genotypes. As stated in the specification on page 15 lines 1-16, the genotype of a plant can be examined using various laboratory techniques. With different genotypes the inbred lines will perform differently and have different combining abilities with various other inbreds. It is this difference in combining ability that is unpredictable and yet so necessary to achieve if improved hybrids are to be developed. On page 335 of Breeding Field Crops (Poehlman et al., 1988) (A9) it states, "After an inbred line of corn is developed, it must be crossed with another inbred line to produce the single-cross hybrid that is grown by the farmer. It is not possible to predict from visual observation which combination of inbred lines will produce productive hybrids. For this reason inbred lines are evaluated for general combining ability using test crosses and for specific combining ability using diallel crosses."

In the instant application, the Examiner has noted some similarities in the morphologies inbred maize line PH48V and the Arthur inbred maize line LH281. However, in addition to these similarities, there are also notable differences, as is documented below.

The following table notes some of the differences between inbred maize line PH48V and the maize line LH281. This information can be found in Table 1 on pages 17-19 of the specification and in columns 5-6 of the Arthur patent, 5,723,739. The Applicant would like to particularly point out the differences in the Northern Leaf Blight ratings and staygreen ratings.

PH48V	LH281
78 days from emergence to 50% plants in silk	84 days from emergence to 50% plants in silk
76 days from emergence to 50% plants in pollen	85 days from emergence to 50% plants in pollen
1534.7 heat units from emergence to 50% plants in silk	1524 heat units from emergence to 50% plants in silk
1486.7 heat units from emergence to 50% plants in pollen	1549 heat units from emergence to 50% plants in pollen
226.7 cm = plant height	242.8 cm = plant height
86.0 cm = ear height	95.8 cm = ear height
51.5 cm = tassel length	35.6 = tassel length
8 = husk tightness rating	5 = husk tightness rating
8-10 cm = husk extension	<8 cm = husk extension
Cob color is red	Cob color is white
7 = Gray Leaf Spot resistance rating	5 = Gray Leaf Spot resistance rating
7 = Northern Leaf Blight resistance rating	8 = Northern Leaf Blight resistance rating
7 = Southern Leaf Blight resistance rating	5 = Southern Leaf Blight resistance rating
8 = staygreen rating	7 = staygreen rating
Aleurone color is yellow	Aleurone color is white
Weight per 100 kernels = 27 gm	Weight per 100 kernels = 24 gm

Applicant would like to point out that the PH48V-derived inbred maize line is not the same product as LH281 therefore the product-by-process claim is not properly rejected over prior art teaching the same product produced by a different process. Applicant further submits that PH48V possesses unique combinations of traits that confer unique combinations of genetics. The number of generations the claimed plant resides from the starting material is not a factor, as long as the end product was developed by use of the invention and expresses a combination of at least two of the essential elements or characteristics unique to inbred line PH48V. What are being claimed are not the traits but the unique combinations of genetics that are passed through the generation(s) and when expressed allow the trait to be expressed. And as previously stated, these unique combinations derived from inbred PH48V can be identified using laboratory techniques such as RFLPs (page 15, lines 1-16 of the specification).

In light of the above, Applicant respectfully requests the Examiner reconsider and withdraw the rejection to claims 14, 17, 33, 36, and 46 under 35 U.S.C. §§ 102(b) and 103(a).

REJECTIONS UNDER 35 U.S.C. §§ 103

The Examiner rejects claims 1-49 under 35 U.S.C. § 103 (a) as obvious over Arthur (U.S. Patent 5,723,739). The Examiner states, "Arthur teaches a maize Dent inbred with absent anthocyanin in the brace roots, dark green leaves, no leaf sheath pubescence, light green glumes, light green silks, upright ear, strong staygreen, high yield, and high resistance to Northern Leaf Blight; wherein said inbred was derived by crossing other plants with desirable agronomic characteristics, and also teaches tissue culture and the use of the inbred in hybrid seed production (see, e.g. columns 5-7).

Arthur does not teach a maize plant with slightly curved kernel rows or red cob.

It would have been obvious to one of ordinary skill in the art to utilize the maize inbred taught by Arthur, and to modify that inbred by crossing with other maize plants to incorporate desired agronomic characteristics, as suggested by the reference."

The Applicant respectfully disagrees with the Examiner. Applicant submits that though PH48V and LH281 exhibit some similar physiological and morphological traits, PH48V is clearly differentiated from LH281 (see previous table). One would not be able to obtain PH48V through modification of the maize inbred taught by Arthur because PH48V comprises an unique and nonobvious combination of previously unknown and nonobvious genetics. Further, plants derived from PH48V are also clearly differentiated. It must be recognized that the PH48V-derived plants are themselves unusual and a nonobvious result of a combination of previously unknown and nonobvious genetics. Thus, they deserve to be considered new and nonobvious compositions in their own right as products of crossing when PH48V is used as a starting material.

In light of the above, Applicant respectfully requests the Examiner reconsider and withdraw the rejection to claims 1-49 under 35 U.S.C. §§ 103(a).

CONCLUSION

Attached hereto is a marked-up version of the changes made to the specification and claims by current amendment. The attached page is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**".

Applicant submits that in light of the foregoing amendments and remarks, the claims as amended are in condition for allowance. Reconsideration and early notice of allowability is respectfully requested. If it is felt that it would aid in prosecution, the

Examiner is invited to contact the undersigned at the number indicated to discuss any outstanding issues.

Respectfully submitted,
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims

3. (Amended) The maize plant of claim 2, wherein said plant [is] has been manipulated to be male sterile.

5. (Amended) A tissue culture according to claim 4, [the] cells or protoplasts of the tissue culture being from a tissue selected from the group consisting of leaves, pollen, embryos, roots, root tips, anthers, silks, flowers, kernels, ears, cobs, husks, and stalks.

14. (Amended) A maize plant, or parts thereof, wherein at least one ancestor of said maize plant is the maize plant of claim 2, said maize plant expressing a combination of at least two traits which are not significantly different from PH48V when determined at a 5% significance level and when grown in the same environmental conditions, said traits selected from the group consisting of: a [relative] maturity of [approximately] 121 based on the Comparative Relative Maturity Rating System for harvest moisture of grain, [high] resistance to Southern Leaf Blight, [high] resistance to Northern Leaf Blight, [high] resistance to Gray Leaf Spot, [high] yield, [above average] resistance to stalk lodging, [above average] resistance to root lodging, [strong] staygreen, [tall] plant height, [high] ear placement and [adapted] adaptability to the Southeast region of the United States.

15.(Amended) A method for developing a maize plant in a maize plant breeding program [using plant breeding techniques, which include employing a maize plant, or its parts, as a source of plant breeding material,] comprising: obtaining the maize plant, or its parts, of claim 2 [as a source of said breeding material.] ;and employing said plant or parts as a source of breeding material using plant breeding techniques.

16.(Amended) The [maize plant breeding program] method of claim 15 wherein plant breeding techniques are selected from the group consisting of: recurrent selection, backcrossing, pedigree breeding, restriction fragment length polymorphism enhanced selection, genetic marker enhanced selection, and transformation.

19. (Amended) The maize plant, or parts thereof, wherein the one or more single gene conversions [conversion(s)] of claim 18 [, wherein the gene is] comprise a dominant allele.

20. (Amended) The maize plant, or parts thereof, wherein the one or more single gene conversions [conversion(s)] of claim 18 [, wherein the gene is] comprise a recessive allele.

22.(Amended) The maize plant of claim 21, wherein said plant [is] has been manipulated to be male sterile.

24. (Amended) A tissue culture according to claim 23, [the] cells or protoplasts of the tissue culture being from a tissue source selected from the group consisting of leaves, pollen, embryos, roots, root tips, anthers, silks, flowers, kernels, ears, cobs, husks, and stalks.

33. (Amended) A maize plant, or parts thereof, wherein at least one ancestor of said maize plant is the maize plant of claim 21, said maize plant expressing a combination of at least two traits which are not significantly different from PH48V when determined at a 5% significance level and when grown in the same environmental conditions, said traits selected from the group consisting of: a [relative] maturity of [approximately] 121 based on the Comparative Relative Maturity Rating System for harvest moisture of grain, [high] resistance to Southern Leaf Blight, [high] resistance to Northern Leaf Blight, [high] resistance to Gray Leaf Spot, [high] yield, [above average] resistance to stalk lodging, [above average] resistance to root lodging, [strong] staygreen, [tall] plant height, [high] ear placement and [adapted] adaptability to the Southeast region of the United States.

34.(Amended) A method for developing a maize plant in a maize plant breeding program [using plant breeding techniques, which include employing a maize plant, or its parts, as a source of plant breeding material,] comprising: obtaining the maize plant, or its parts, of claim 21 [as a source of said breeding material.] ;and employing said plant or parts as a source of breeding material using plant breeding techniques.

35.(Amended) The [maize plant breeding program] method of claim 34 wherein plant breeding techniques are selected from the group consisting of: recurrent selection, backcrossing, pedigree breeding, restriction fragment length polymorphism enhanced selection, genetic marker enhanced selection, and transformation.

37. (Amended) A process for producing inbred PH48V, representative seed of which have been deposited under ATCC Accession No. _____, comprising:

(a) planting a collection of seed comprising seed of a hybrid, one of whose parents is inbred PH48V, said collection also comprising seed of said inbred;

(b) growing plants from said collection of seed;

(c) identifying [said] inbred [PH48V] parent plants;

(d) selecting said inbred [PH48V] parent plant; [and]

(e) controlling pollination [in a manner] through selfing which preserves the homozygosity of said inbred [PH48V] parent plant[.]; and

(f) collecting morphological and/or physiological data so that said inbred parent may be identified as inbred PH48V.

41. (Amended) A PH48V-derived maize plant, or parts thereof, produced by the method of claim 40, said PH48V-derived maize plant expressing a combination of at least two traits which are not significantly different from PH48V when determined at a 5% significance level and when grown in the same environmental conditions, said traits selected from the group consisting of: a [relative] maturity of [approximately] 121 based on the Comparative Relative Maturity Rating System for harvest moisture of grain, [high] resistance to Southern Leaf Blight, [high] resistance to Northern Leaf Blight, [high] resistance to Gray Leaf Spot, [high] yield, [above average] resistance to stalk lodging, [above average] resistance to root lodging, [strong] staygreen, [tall] plant height, [high] ear placement and [adapted] adaptability to the Southeast region of the United States.

43. (Amended) [A] The further [derived] PH48V-derived maize plant, or parts thereof, produced by the method of claim 42.

45. (Amended) A PH48V-derived maize plant, or parts thereof, produced by the method of claim 44, said PH48V-derived maize plant expressing a combination of at least two traits which are not significantly different from PH48V when determined at a 5% significance level and when grown in the same environmental conditions, said traits selected from the group consisting of: a [relative] maturity of [approximately] 121 based on the Comparative Relative Maturity Rating System for harvest moisture of grain, [high] resistance to Southern Leaf Blight, [high] resistance to Northern Leaf Blight, [high] resistance to Gray Leaf Spot, [high] yield, [above average] resistance to stalk lodging, [above average] resistance to root lodging, [strong] staygreen, [tall] plant height, [high] ear placement and [adapted] adaptability to the Southeast region of the United States.

46.(Amended) The further PH48V-derived maize plant, or parts thereof, of claim 43, wherein said further PH48V-derived maize plant, or parts thereof, express a combination of at least two traits which are not significantly different from PH48V when determined at a 5% significance level and when grown in the same environmental conditions, said traits selected from the group consisting of: a [relative] maturity of [approximately] 121 based on the Comparative Relative Maturity Rating System for harvest moisture of grain, [high] resistance to Southern Leaf Blight, [high] resistance to Northern Leaf Blight, [high] resistance to Gray Leaf Spot, [high] yield, [above average] resistance to stalk lodging, [above average] resistance to root lodging, [strong] staygreen, [tall] plant height, [high] ear placement and [adapted] adaptability to the Southeast region of the United States.

47. (Amended) The maize [plants] plant, or parts thereof, of claim 21, further comprising one or more single gene conversions.

48. (Amended) The maize plant, or parts thereof, wherein the one or more single gene conversions [conversion(s)] of claim 47 [, wherein the gene is] comprise a dominant allele.

49. (Amended) The maize plant, or parts thereof, wherein the one or more single gene conversions [conversion(s)] of claim 47 [, wherein the gene is] comprise a recessive allele.

New claims 50 and 51 have been added.

PRINCIPLES OF CULTIVAR DEVELOPMENT

VOLUME 1

Theory and Technique

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Iowa State University

with the assistance of
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CHAPTER NINETEEN

Field-Plot Techniques

The fundamental purpose of plant breeding is to identify genotypes with superior performance in commercial production. A large proportion of the time and expense devoted to cultivar development is in field evaluation of breeding material. The tests may involve genotypes in an initial stage of evaluation or those being given final consideration for release as new cultivars. The characters evaluated range from those that can be measured readily by visual examination to those that must be measured with appropriate instruments. The genetic potential of a genotype for some characters may be determined effectively with one or a few plants in a small plot, while for other characters extensive evaluation in larger plots may be needed.

It is the responsibility of the plant breeder to select the field-plot techniques that will provide the maximum amount of information with the resources available. The challenge is to adequately test as many genotypes as possible. The resources available to plant breeders vary; usually several alternative techniques are available for character evaluation. Plant breeders must decide which techniques will be the most effective and efficient in their particular situation.

Detailed discussions of field-plot techniques and data analysis are provided by Gomez and Gomez (1984) and LeClerg et al. (1962). An overview of the general principles will be provided in this chapter.

SOURCES OF VARIATION

The ideal way to compare genotypes would be to grow all of them in exactly the same environment and to measure their characteristics in precisely the same manner. The differences among genotypes in this ideal situation would be due only to variation in their genetic potential; therefore, the best genotype could be chosen without error. This ideal is impossible to achieve under field conditions because of lack of uniformity in the environment to which the genotypes are

exposed. Nevertheless, the use of appropriate field-plot techniques can maximi the accuracy with which genotypes are compared and selected.

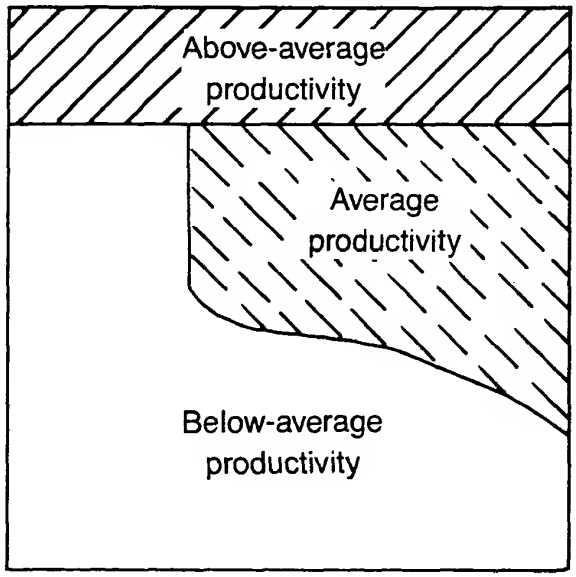
The factors that result in test conditions that are less than ideal can be refer to collectively as sources of experimental error. They include variation in t environment to which each genotype is exposed and lack of uniformity in t measurement of characters. The breeder has opportunities to minimize exper mental error by carefully selecting the site to be used for field trials, the cultu practices used in crop production, the plot size and shape, and the method o data collection.

Site Selection

Variation in the productivity of the soil is commonly referred to as soil heterogeneity (Fig. 19-1). Causes of soil heterogeneity include variation in soil type, availability of plant nutrients, and soil moisture. The variation cannot be completely eliminated, but it often can be minimized by careful selection of the area in a field where plots will be grown. Soil maps are helpful for understanding the variation in soil type that is present. Soil types differ in their inherent ability to retain nutrients and moisture. Entire trials or at least an entire replication should be grown on a single soil type whenever possible.

Visual inspection of a field is important, even when a soil map is available.

Figure 19-1 Example of potential variation in soil productivity in a test area.



When a field has been identified a year in advance as a potential test site, it is useful for the breeder to look for variability in productivity of the crop grown in the area. The breeder should note variation in the terrain that may cause water to accumulate more in one place than in another. Differences in soil tillage after harvest of the previous crop may be observed that could result in nonuniformity of the area. Uneven distribution of plant or animal waste on a field should be noted as a potential contributor to variation in the availability of plant nutrients.

Before a site is chosen, information should be obtained on cultural practices that were followed in the production of previous crops, with special attention to the application of chemicals that could influence the crop that the breeder will be evaluating. The residue from herbicides applied for control of weeds in previous crops may cause damage to the crop to be tested. The following quotation from a research article by Thorne and Fehr (1970b) on soybean breeding illustrates the importance of herbicide residue:

> The strains were evaluated at Ames and Kanawha, Iowa, in 1968. . . . At Kanawha, part of the experiment was inadvertently planted in a field treated with atrazine herbicide two years before. All plots in the area were destroyed.

Previous cultural practices in a field can be especially important at research stations where crops are rotated from one field to another on a systematic basis. The research conducted on crops previously grown on a field can influence markedly the uniformity of the test site. For example, plots of oats were planted in a field at the Agronomy Research Center of Iowa State University in which soybeans had been planted the previous year. Growth of the oats varied in strips, as if nitrogen fertilizer had been applied unevenly to the field. A review of the previous soybean research revealed that the strips of oats with extra growth coincided with areas where mature soybeans had been cut and left unthreshed. The nitrogen in the soybean seeds in the strips was available to the oats the following year, and caused nonuniformity of nutrient availability in the test site.

Cultural Practices

Experimental error can be minimized by the use of uniform cultural practices for production of the crop being tested. Chemicals should be applied uniformly to the test site before, during, or after planting. Uneven soil compaction should be minimized during tillage operations. Application of supplemental water by irrigation may reduce variability in soil moisture. Weed control should be uniform; most breeders try to eliminate all weeds during the growing season to avoid experimental error caused by differential weed competition.

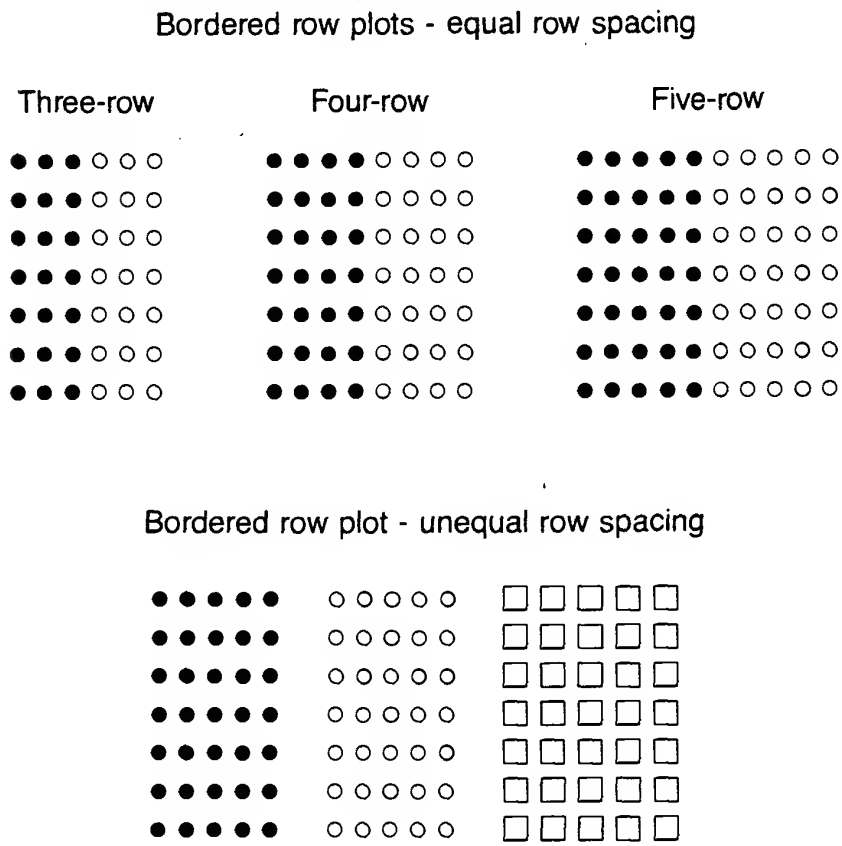
The development of equipment specifically designed for planting, managing, and harvesting research plots has permitted breeders to grow plots more efficiently. The emphasis in the design and use of any equipment must be on the uniformity with which genotypes are handled.

Plot Type

Experimental error increases whenever interplot competition causes the performance of a genotype in one plot to be altered by the performance of genotypes in adjacent plots. Interplot competition results primarily from intergenotypic competition, which is the differential ability of genotypes to compete with each other. Interplot competition is more important for the evaluation of some characters than for others. It is only through appropriate experimentation that a plot type can be identified that will provide reliable information for the character of interest.

The effects of interplot competition can be avoided by the use of plots with multiple rows in which only plants in the center rows are evaluated (Fig. 19-2). In plots with three or more rows, the outermost rows are designated as the border or guard rows. The function of the border rows is to prevent plants in adjacent

Figure 19-2 Illustration of bordered row plots with different cultivars designated as ●, ○, and □. (Courtesy of Fehr, 1978.)

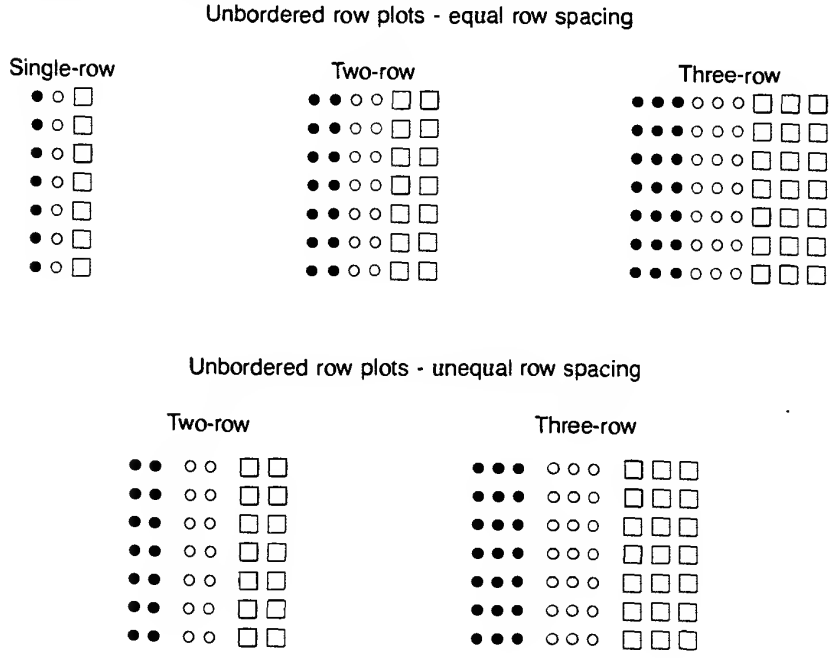


plots from influencing the performance of plants in the center of the plot. Each bordered plot can be considered a miniature field that is unaffected by neighboring fields. The spacing between plots can be greater than the within-plot spacing to facilitate the movement of equipment, particularly when narrow rows are utilized.

It would be ideal if bordered plots could be used for the evaluation of all characters that are influenced by interplot competition. That ideal is difficult to achieve when thousands of genotypes are being evaluated. Bordered plots require seed and land that do not directly provide data for a genotype. Borders take up two-thirds of the seed and land area for three-row plots and one-half for four-row plots. The cost and availability of seed and land often necessitate restriction of the use of bordered plots to the evaluation of genotypes that are being given final consideration for release as cultivars.

Interplot competition can be reduced, but not eliminated, with unbordered plots of two or more rows, all of which are used to evaluate a character (Fig. 19-3). A genotype in a single-row plot is subjected to interplot competition on both sides. Interplot competition is reduced by one-half in plots with two rows, two-thirds with three rows, three-fourths with four rows, and four-fifths with five rows. The estimated reduction of interplot competition with increasing numbers of rows is based on the fact that each row of a plot must compete on two sides. The border rows are each subjected to interplot competition on one side

Figure 19-3 Illustration of unbordered row plots with different cultivars designated as ●, ○, and □. (Courtesy of Fehr, 1978.)



but not on the other. Any rows within the two border rows are protected from interplot competition. This can be expressed as

$$\text{Reduction in interplot competition compared with single-row plot} = \frac{(\text{number of rows per plot} \times 2 \text{ sides}) - 2 \text{ sides}}{\text{number of rows per plot} \times 2 \text{ sides}}$$

$$\text{Two-row plot} = \frac{(2 \times 2) - 2}{2 \times 2} = 1/2$$

$$\text{Three-row plot} = \frac{(3 \times 2) - 2}{3 \times 2} = 2/3$$

The amount of interplot competition also can be reduced by increasing the spacing between rows of adjacent plots. Interplot competition in soybeans was evaluated with five cultivars grown in single rows spaced 100, 75, 50, and 25 cm apart (Gedge et al., 1977). The average effect of interplot competition on seed yield was 2.6 percent for the 100-cm spacing, 5.3 percent for 75 cm, 8.0 percent for 50 cm, and 17.6 percent for 25 cm.

A combination of increased row spacing between plots and a large number of rows can minimize interplot competition in unbordered plots. In the soybean example of the preceding paragraph, the average change in yield for single-row plots spaced 100 cm apart was 2.6 percent. The percentage theoretically would be reduced to 1.3 percent for two-row plots and to 0.9 percent for three-row plots. Rows within a plot are not subjected to interplot competition; therefore, the spacing between rows within a plot can be less than the spacing between adjacent plots. Figure 19-3 illustrates a two-row plot in which the spacing between plots is wide enough to minimize interplot competition and the spacing within the plot is reduced to minimize the land area required for each plot.

Some breeders plant one cultivar as a common border between one- or two-row plots. In barley, a lodging-resistant cultivar is used as a common border to prevent genotypes with lodging susceptibility from falling on genotypes in adjacent plots, thereby causing them to lodge unnaturally. The use of a common border has been evaluated as a means of eliminating intergenotypic competition between plots for seed yield and other quantitative characters. The results of the research indicate that a common border can reduce but not eliminate interplot competition (Thorne and Fehr, 1970a). The average interplot competition for seed yield among four soybean cultivars in single-row plots spaced 50 cm apart was compared with competition of the cultivars when a common border was used (Gedge et al., 1977). Interplot competition averaged 11.0 percent in single-row plots and 8.3 percent in plots with a common border.

Plot Size and Shape

The size of plots used to evaluate genotypes varies with the character being evaluated, the amount of experimental error that is considered acceptable for

measuring a character, the experimental design, and the growth characteristics of the crop. Plots vary in size from those for a single plant that is harvested by hand to those that are wide and long enough to be harvested with the same equipment used by farmers for commercial production.

Single-Plant Plots. Individual plants commonly are evaluated in segregating populations. There is no replication of the individuals, unless vegetative propagation of clones is possible. The spacing among plots varies with the crop species involved. Gardner (1961) spaced individuals 50 by 100 cm apart when selecting for yield in maize. Burton (1974) spaced plants of a population of Pensacola bahiagrass 60 by 60 cm apart when conducting recurrent phenotypic selection for forage yield. Burton and Brim (1981) used a 46 by 46 cm spacing among soybean plants for selection of oil composition in the seed.

Single-plant plots are used for the replicated evaluation of experimental lines or cultivars by the honeycomb field design (Fasoulas, 1979). The number of plants evaluated for a line is equal to the number of replications in the experiment. Fasoulas (1981) indicated that 100 single-plant plots (replications) per line would provide satisfactory results. The plots of the lines in a test are organized in a systematic manner to permit comparison of a plant of one line with adjacent plants of other lines (Fig. 19-4). The honeycomb design has not been adopted by plant breeders for replicated evaluation of lines because it requires more labor and is less amenable to mechanization than microplots or conventional row plots.

Multiple-Plant Plots. The evaluation of experimental lines or cultivars by plant breeders is usually done in plots containing two or more plants. Plot size varies from small microplots consisting of a hill or short row to a plot with one or more rows several meters in length.

Microplots. Microplots are used to minimize the amount of seed or land required to evaluate a group of lines. In an unbordered microplot, the effects of interplot competition must be considered when determining an appropriate distance among plots (Fig. 19-5). For oats, hill plots spaced about 30 by 30 cm apart have been used (Frey, 1965), while for soybeans, a spacing of about 1 by 1 m is more common (Garland and Fehr, 1981).

The number of plants in a microplot differs among crops. A planting rate of 30 seeds per hill is satisfactory in oats (Frey, 1965), while a rate of 12 seeds per hill is used for soybeans (Garland and Fehr, 1981). When short rows are used as microplots, the plant density is comparable to that of larger row plots.

There is a lack of agreement among plant breeders concerning the effectiveness of microplots for evaluation of agronomic characters, particularly seed yield. Breeders who use microplots indicate that they are useful for eliminating inferior lines during the first year of yield evaluation. Lines with acceptable performance in microplots are evaluated in conventional row plots during subsequent years of testing, to identify those that merit release as cultivars (Frey,

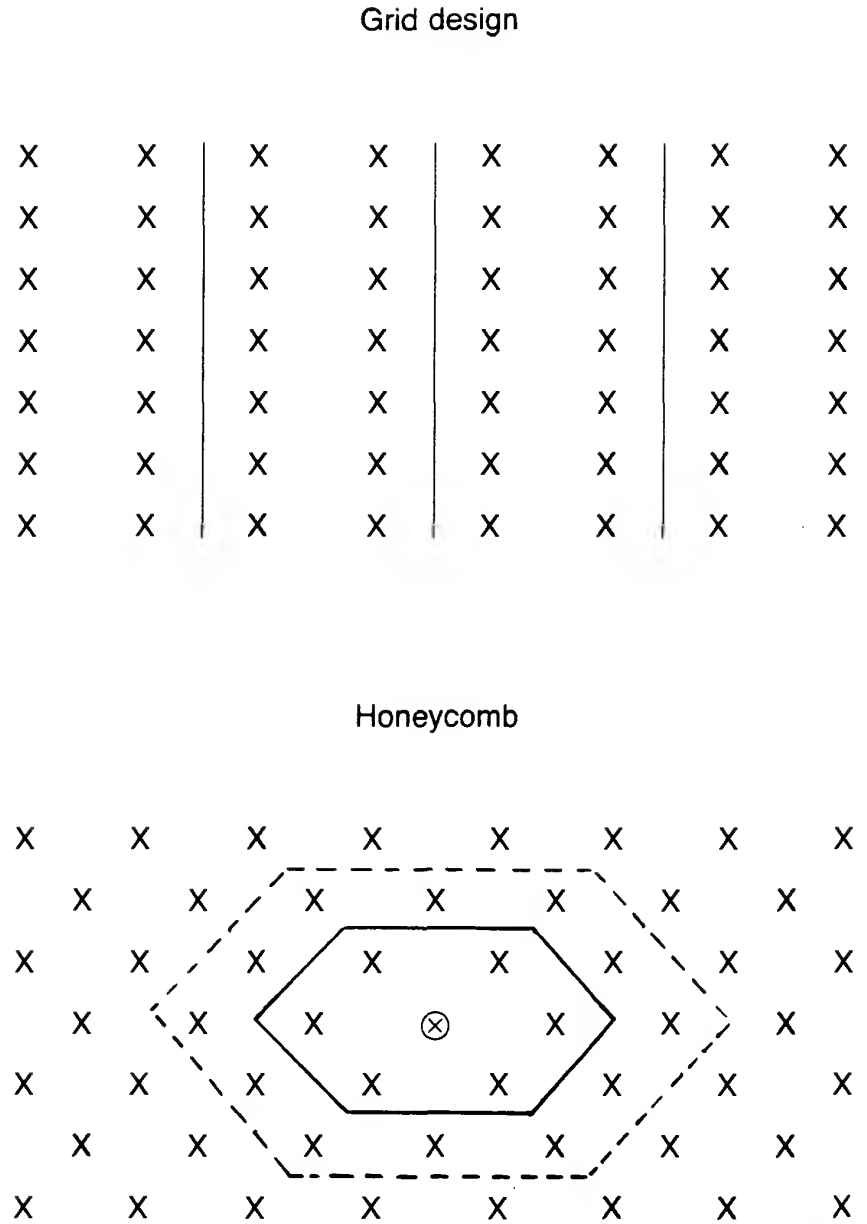


Figure 19-4 Grid and honeycomb design to select individual plants in a population. For the grid design, plants are divided into blocks and the best ones chosen from each (Gardner, 1961). For the honeycomb design, the plant at the center of the hexagon, ⊗, is compared with every other plant within the hexagon (Fasoulas, 1979). A plant is chosen only if it is superior to every other plant in the hexagon. The hexagons outlined represent two different selection intensities.

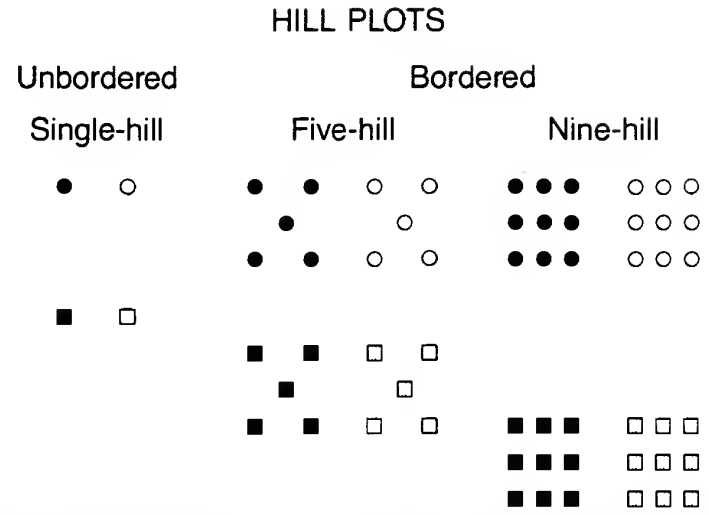


Figure 19-5 Illustration of hill plots with different cultivars designated as □, ○, ●, and ■ (Fehr, 1978).

1965; Garland and Fehr, 1981). The advantages of microplots compared with conventional row plots for the first year of yield testing are that less land is required per plot and that enough seed for replicated tests can be obtained from a single plant, which eliminates a season for seed increase. Breeders who do not use microplots are concerned about the reliability of yield data obtained from them. The coefficients of variability for microplots generally are about one and one-half to two times larger than for conventional row plots.

Row Plots. Row plots are used by virtually all plant breeders for replicated testing of genotypes. The overall plot size is determined by the number of rows, the spacing between rows, and the row length.

Single-row plots of 1 to 2 m in length are widely used for the visual evaluation of characters. Many breeders evaluate lines on the basis of their appearance in small unreplicated plots, and advance the desirable ones to replicated tests the following season. Visual selection and seed increase commonly are accomplished with the same plot.

A plot used to evaluate the yield of lines for the first time often is smaller than that employed for advanced stages of evaluation. For advanced yield tests, the breeder attempts to use a plot size that approaches or equals the dimensions considered optimal for the crop species involved. Optimum plot size is the minimum land area required to measure a character with an acceptable level of experimental error.

Optimum plot size can be determined by the use of data from a uniformity trial (Cochran, 1937). A single cultivar is planted as a solid stand, without alleys,

in an area representative of that used for yield evaluation. The cultural practices used to produce the crop are the same as those used for yield trials. The area is subdivided into small units, and the seeds or plants from each unit are harvested and weighed separately. Experimental error associated with plots of different size can be determined by making various combinations of the small units.

Optimum plot size also is determined through practical experience. The breeder often will experiment with plots of different size to find the smallest one that has an acceptable level of experimental error. Breeders often do not agree on what they consider acceptable experimental error; consequently, an optimum size for one person may not be optimum for another.

Plot width generally is determined by considerations other than the relationship of shape to experimental error. The primary factors are the number of rows required to minimize or avoid interplot competition and the width of the planting and harvesting equipment that is available. Plot width influences the percentage of land area that must be devoted to alleys between plots. Long, narrow plots require a lower percentage of alley space than do wide, short plots. This advantage is offset in bordered plots because the percentage of land area devoted to border rows decreases as the number of rows per plot increases.

Plot length provides flexibility for plot size. Before calculators and computers became readily available, row length in the United States was varied to obtain a plot size that was a fraction of an acre (one-tenth, one-twentieth, etc.) to simplify the conversion of plot yields to yields per acre. With use of computers for data summarization and analysis, this is no longer necessary.

Data Collection

The experimental error associated with the evaluation of a character is influenced by measurement errors during data collection. For characters evaluated visually, experimental error occurs whenever the data collector fails to give an identical rating to plots with an identical appearance. Reliability of the evaluation can be established readily by rating a series of plots at different times and comparing the ratings. It is essentially impossible to give visual ratings without error; therefore, the breeder must decide when the error is acceptable and when it is so large that genetic differences will be masked.

Some characters can only be evaluated efficiently with the use of an appropriate machine or instrument. Experimental error can occur because of failure to prepare a plot properly for measurement, of not obtaining a representative sample of the plot for evaluation, of using nonuniform procedures for sample preparation, and of failure of the machine or instrument to operate properly.

Preparation of a plot for data collection may begin before planting. For experimental error to be reduced, the seeds or plants of every genotype used for planting must be treated equally. If seeds or plants of genotypes to be compared

do not come from a common environment, environmental error may result. Lint yield and seedling vigor of a cotton cultivar were found to differ in plots grown from seeds obtained from different locations (Peacock and Hawkins, 1970). Seed source also has been shown to influence seed yield of soybeans (Fehr and Probst, 1971.)

In some crop species, uniformity of plant density among plots can be important in minimizing experimental error. With maize, it is a common practice to thin yield test plots to a uniform stand soon after seedling emergence. Thinning is not considered necessary with some crop species, particularly those that have the ability to branch or tiller in response to low plant density, such as barley and wheat. It also is a common practice with crops such as maize to record the number of plants per plot immediately before harvest. The yield of the plots is adjusted for plant density by an analysis of covariance, to minimize experimental error in the comparison of genotypes.

When a blank alley is used at the end of row plots, the end plants generally are more productive than those growing in the center of the plot. When end plants are harvested, yield of the plot is inflated in comparison to the yield obtained from plants growing in the center of the plot. This inflation will prevent a direct comparison of plot yields with those expected in a normal commercial planting, unless an appropriate adjustment is made for all plots. The adjustment may be made by considering the alley as part of the plot area; therefore, plot length is the distance from the center of one alley to the center of the next, instead of the distance between plants at opposite ends of a row. For example, if the length of row containing plants is 5 m and the alley is 1 m wide, the plot length for computing plot area is considered to be 6 m.

The yield inflation by end plants in a plot does not contribute to experimental error unless genotypes in a test do not respond similarly to the space in the alley. The experimental error associated with differential response of genotypes to an alley can be minimized by adjusting yields according to characteristics of the genotypes that influence this response. The end plants of soybean genotypes with late maturity give a greater yield inflation than do genotypes of early maturity. Values have been developed with which to adjust plot yields for maturity of soybean genotypes (Wilcox, 1970). More commonly, comparisons among soybean genotypes are restricted to those of similar maturity, unless plots are end-trimmed before harvest.

The only way to eliminate yield inflation by end plants is to remove the plants before harvest. This procedure, referred to as end-trimming, is a standard procedure with some crops. The end plants are removed late enough in plant development that the remaining plants in the plot cannot take advantage of the extra space. The length of row removed from each end of the plot must be long enough to include all plants that have benefited from the space provided by the alley. In soybean, 0.6 m is removed from each end of the plot (Wilcox, 1970).

The problem of a blank alley is minimized in some crops by planting the

alley with rows of a single genotype perpendicular to the test plots. The result is that the plants at the end of a plot must compete with plants in the alley, and thus their yield may not be inflated as much as is the case with a blank alley. Plants in the alley are removed immediately before the plots are harvested.

EXPERIMENTAL DESIGNS

The arrangement of genotypes in a field experiment is referred to as the experimental design. Some of the designs utilized to compare genotypes are common to research in many disciplines. Others have been developed to deal with the problem of comparing a large number of genotypes as inexpensively as possible. The experimental designs used for the initial evaluation of a large number of genotypes often differ from those used in the advanced stages of testing a few select genotypes. Alternative designs will be considered here for comparison of single plants, unreplicated genotypes in multiple-plant plots, and replicated genotypes.

Single-Plant Selection

The first evaluation step in the development of a cultivar generally is the selection of individual plants from a population. Individual plant selection also is employed in population improvement by recurrent phenotypic selection.

When single-plant selection in a population is for characters with a high heritability, the plants generally are grown in a random order and those with desirable characteristics are selected. Cultivars may be grown in adjacent plots to serve as standards with which to evaluate single plants. Date of flowering, plant height, time of maturity, and certain types of pest resistance are examples of characters for which single plants are selected without any predetermined arrangement of the individuals. They represent characteristics that are not strongly influenced by environmental variation.

Single-plant selection in a population grown in a relatively large land area can be hampered seriously by soil heterogeneity for characters with a low heritability, such as seed or plant yield. Figure 19-1 illustrates variation in soil productivity in an area where a population of plants may be grown. If plants with the highest yield are selected regardless of their location in the field, those in the area of above-average productivity will be favored. A plant with outstanding genetic potential that is located in the area with below-average productivity may be discarded. Two experimental designs are available that minimize the effect of soil heterogeneity by comparing plants that are most adjacent to each other.

Grid Design. Gardner (1961) proposed that the land area on which a population of individual plants is grown can be subdivided into blocks or grids of a limited

area (Fig. 19-4). Plants within each block are compared with each other, and the superior ones are selected. Comparisons are not made between plants from different blocks. This experimental design has been well accepted by plant breeders, particularly those conducting recurrent phenotypic selection for yield or other characters with a low heritability.

Honeycomb Design. Fasoulas (1973) developed a honeycomb design for selecting individual plants in a population (Fig. 19-4). Five aspects of the design and its implementation are unique. (a) Seeds or clones are spaced equidistantly from each other in a hexagon pattern. The name of the design was chosen because the hexagon patterns resemble a honeycomb of bees. (b) Plants are spaced far enough apart that they do not compete with adjacent individuals. At the appropriate spacing for a species, a missing plant does not influence the performance of adjacent individuals, because each plant already has sufficient space in which to develop to its full potential. (c) Homogeneous check cultivars can be included for comparison, if desired. Every plant of the check is compared with a different group of plants in the population. (d) The size of the hexagon used to select single plants determines the selection intensity in the population. The effect of soil heterogeneity is minimized because only those plants within the area of the hexagon are compared. (e) Every plant in the population is evaluated by placing it in the center of the hexagon. A plant is chosen only if it is superior to every other plant in the hexagon. By moving the hexagon, every plant is compared with a different group of plants in the population.

Comparison of the Grid and Honeycomb Designs. Both the grid and honeycomb designs reduce the problem of soil heterogeneity in the selection of characters of low heritability. In a comparison of the designs, the advantages of one are the disadvantages of the other, and vice versa.

There are three primary advantages of the grid design.

1. The spacing of plants does not have to be in a precise pattern. This facilitates the use of conventional plot equipment for planting and cultivation. Mechanized planting of the honeycomb design would require specialized equipment.
2. Selection intensity can be varied by altering the number of plants in a block and the number of plants selected. Only certain selection intensities are possible with the honeycomb design.
3. Use of a defined area for each block facilitates visual comparison of plants for selection. It is possible to compare plants within a block visually and collect data only from those with the best potential. Use of the moving hexagon for the honeycomb design makes it impractical to compare each plant with appropriate ones in its hexagon; therefore, data must be recorded for every plant, except those that are obviously inferior.

The honeycomb design has two advantages compared with the grid design.

1. Homogeneous check cultivars can be included to permit comparisons of individual plants with a standard. When one-seventh of the plants are a check, they can be arranged so that every plant in the population can be compared with a check plant. To provide adjacent plants of one check cultivar in a grid system, one-third of the area would have to be devoted to the check.
2. More than two check cultivars can be included readily in hexagons of 19 or more plants. Use of two or more check cultivars in the grid system would require that a large fraction of each block be devoted to check plants.

Unreplicated Evaluation with Multiple-Plant Plots

Plant breeders routinely conduct visual selection among lines in unreplicated plots for maturity, disease resistance, standability, and other characters of high heritability. Evaluation for yield in a single replication has been used to a limited extent to eliminate inferior lines before initiation of expensive replicated tests. With a single replication, each line is compared once with check cultivars or other lines to determine its genetic potential. A number of different arrangements are available for estimating the genetic potential of lines. One method is to compare each line with a common check cultivar (Baker and McKenzie, 1967). Figure 19-6 represents a hypothetical example of the yield of six lines in a single replication. In the figure, the yield of each line is expressed as a percentage of the yield of the check cultivar immediately adjacent to it.

Another alternative is to express the yield of each line as a percentage of the weighted average of the adjacent check plot and of the check plot two plots removed. The purpose for using a weighted average is to minimize the potential problem caused by an unusually poor yield of a check plot. In Fig. 19-6, the check cultivar adjacent to lines B and C has a much lower yield than other check cultivars. This results in an extremely high percentage for lines A and B. The weighted average of check cultivars could be computed as

$$(\tfrac{2}{3} \times \text{yield of adjacent check}) + (\tfrac{1}{3} \times \text{yield of check two plots removed}) = \text{weighted average of check cultivars}$$

The percentage yield of each line is computed as

$$\text{Line A} = \frac{59}{(\frac{2}{3} \times 55) + (\frac{1}{3} \times 39)} \times 100 = 119$$

$$\text{Line B} = \frac{70}{(\frac{2}{3} \times 39) + (\frac{1}{3} \times 55)} \times 100 = 158$$

$$\text{Line C} = \frac{53}{(\frac{2}{3} \times 39) + (\frac{1}{3} \times 48)} \times 100 = 126$$

$$\text{Line D} = \frac{51}{(\frac{2}{3} \times 48) + (\frac{1}{3} \times 39)} \times 100 = 113$$

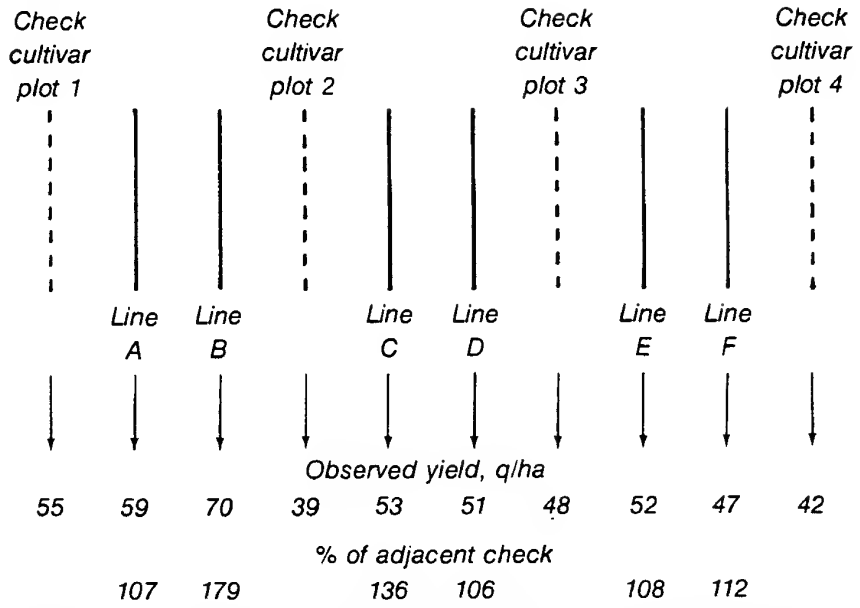


Figure 19-6 One possible arrangement of lines in a single-replication test. The performance of each line is computed as a percentage of the performance of the common check cultivar adjacent to it. Line B would be considered the superior one.

$$\text{Line E} = \frac{52}{(\frac{2}{3} \times 48) + (\frac{1}{3} \times 42)} \times 100 = 113$$

$$\text{Line F} = \frac{47}{(\frac{2}{3} \times 42) + (\frac{1}{3} \times 48)} \times 100 = 107$$

Another method used to compare genotypes in single replications is the moving mean (Mak et al., 1978; Townley-Smith and Hurd, 1973). Each genotype is compared with adjacent test genotypes, not with a check cultivar.

The disadvantage of single-replication tests is that the breeder has only one plot value with which to assess the genetic potential of a line. If by chance a line is placed on a plot of soil with above-average productivity, relative to that of plots with which the line is compared, it will seem to be genetically superior, even though it may not be. In replicated tests, the breeder will have more than one plot with which to evaluate each line. For this reason, single replications are not commonly used for yield evaluation.

Replicated Tests

Two or more independent comparisons of lines in a test provide a means of estimating whether variation in performance among lines is due to differences in genetic potential or to environmental variation. Each comparison is as rep-

lication. Replication can be accomplished by growing two or more plots of each line at one or more locations or one plot at each of two or more locations or years.

Randomization. One important consideration in the arrangement of genotypes within each replication is the degree of randomization. From a statistical viewpoint, randomization of entries is required to obtain a valid estimate of experimental error. To fulfill the requirement, each entry must have an equal chance of being assigned to any plot in a replication and an independent randomization is required for each replication.

Plant breeders understand the importance of randomization and consider it the ideal procedure for comparison of genotypes. They know that any experiment designed to estimate components of variance must be randomized. There are circumstances, however, in which plant breeders do not use complete randomization for the comparison of genotypes. Genotypes with similar characteristics may be planted next to each other to reduce interplot competition in unbordered plots. A nonrandom arrangement of genotypes among replications may be used to facilitate selection of genotypes before harvest.

Nonrandom Arrangements of Genotypes. Any discussion of nonrandom arrangements of genotypes can be misinterpreted because it may imply that randomization is not an important principle. To avoid such misinterpretation, it should be stated again that nonrandomization should only be considered when resources are not adequate to make randomization feasible. The discussion of nonrandom arrangements will include the reasons for their use, their disadvantages, and the ways procedures can be modified to permit effective randomization.

Nonrandomization Among Replications. It is common to delay replicated tests for yield until genotypes have been visually selected in unreplicated plots for characteristics such as lodging, height, and maturity. To reduce the length of time for cultivar development, the season for evaluation in unreplicated plots can be eliminated by growing genotypes in replicated plots, visually selecting those with desirable characteristics, and harvesting only the plots of selected genotypes for yield evaluation (Garland and Fehr, 1981). When visual selection is based on the performance of genotypes in all of the replications, it is necessary to evaluate each plot, summarize the data, make the selections, and identify the plots of selected genotypes that should be harvested. The length of time between plot evaluation and harvest may be only a few days when characteristics of interest are not expressed until plant maturity. If several thousand genotypes are randomized in two or more replications, summarization of data and identification of plots to be harvested can be difficult or impossible to accomplish in only a few days. The use of the same arrangement of genotypes in each replication makes the job practical.

When genotypes are in the same position within each replication, the data for plots of each genotype are recorded in adjacent columns (Fig. 19-7). Sum-

Nonrandom

Plot	Entry	Replication 1	2	3
1	1			
2	2			
3	3			
4	4			
5	5			
6	6			

Random

Plot	Entry	Replication 1
1	4	
2	1	
3	6	
4	3	
5	5	
6	2	

Plot	Entry	Replication 2
1	5	
2	4	
3	2	
4	1	
5	6	
6	3	

Plot	Entry	Replication 3
1	2	
2	6	
3	3	
4	5	
5	1	
6	4	

Figure 19-7 Field book pages for recording the data of genotypes grown in three replications. Nonrandom arrangement of genotypes involves one page, whereas a random arrangement involves three separate sections on one or more pages.

marization of data is complete as soon as the last plot is rated. Genotypes with undesirable characteristics in one or more replications can be identified and discarded. The plots of desirable genotypes are readily identified for harvest because they are in the same position in each replication.

The disadvantages of nonrandomization relate to the fact that the same genotypes are always adjacent to each other, which can have negative effects on the comparison of genotypes.

1. In unbordered plots, intergenotypic competition can bias the performance of genotypes more seriously in a nonrandom than in a random arrangement. When a poor competitor is bordered by a good competitor, yield of the poor competitor can be reduced and that of the good competitor increased in every replication. There is no opportunity for a genotype to occur next to others with a more similar competitive ability.
2. In unbordered plots, a genotype that dies or is unusually weak in all replications can prevent the accurate evaluation of adjacent genotypes. The performance of adjacent genotypes would never be tested in replications where they were next to healthy genotypes.
3. No unbiased estimate of experimental error can be obtained.

The need to use nonrandomization of genotypes among replications can be avoided by improving the efficiency of procedures for data summarization and evaluation. An efficient procedure would include the use of a computer. Data would have to be entered rapidly into the computer, possibly by entering plot data into an electronic recorder in the field and electronically transferring the information to the computer. Computer programs would be needed to summarize the data and make selections on the basis of standards established by the breeder. Plot identification information for selected genotypes would have to be provided for harvest.

Grouping Similar Genotypes Within Replications. The evaluation of genotypes in unbordered plots can be hampered by bias from intergenotypic competition. Plant characteristics that often contribute to intergenotypic competition in a crop include such factors as differences in height and time of maturity. To reduce intergenotypic competition, genotypes with similar characteristics may be grouped within replications. The position of each genotype may be varied from one replication to the next. This procedure, sometimes referred to as restricted randomization, has the advantage of reducing the effects of intergenotypic competition in unbordered plots. The primary disadvantage is that all genotypes in a test cannot be compared with the same level of confidence. Genotypes within a group are spaced closer to each other than genotypes in different groups and are less affected by environmental variation among plots.

The use of bordered plots eliminates the need for grouping genotypes. The performance of genotypes in plots is not influenced by intergenotypic compe-

tition; therefore, randomization is practical. An increase in land, seed, and other resources will be needed for replacement of unbordered plots with bordered ones.

Experimental Designs for Replicated Tests. The arrangement of genotypes in replicated tests involves primarily the use of either the randomized complete-block design or incomplete-block designs. The Latin square is used only in special circumstances when the number of entries is small (Cochran and Cox, 1957). The honeycomb design can be used for replicated testing but is considered too difficult to implement for a large number of lines (Fasoulas, 1981).

The differences between the randomized complete-block and incomplete-block designs relate to their ability to account for environmental variation within a replication. The two types of design differ in restrictions on the size of a replication, randomization procedures, analysis of data, and comparisons among genotypes.

The terms complete-block and incomplete-block refer to the arrangement of genotypes in an experiment (Fig. 19-8). A block and a replication are equivalent in a randomized complete-block design. A block contains all of the genotypes in the test and is considered complete. Genotypes are divided into more than one block within each replication of an incomplete-block design. The blocks are considered incomplete because they contain only part of the genotypes. A number of different types of incomplete-block designs are available (Cochran and Cox, 1957). The most common types used in plant breeding are referred to as lattices. In a lattice design, a replication is divided into blocks that collectively contain all the genotypes in a test (Fig. 19-8).

The incomplete-block designs are intended to provide more control over environmental variation within a replication than is possible with the complete-block design. The ideal situation for genotype evaluation would be to test each genotype in the same plot, thus avoiding any environmental variation caused by differences in soil fertility, moisture, and other factors within a field. This is not possible, so the next best approach is to adjust the performance of each genotype according to the relative productivity of the plot in which it is evaluated. If one plot has better fertility and moisture than the average for all plots in a replication, the performance of a genotype in that plot will be adjusted downward. A genotype in a plot with lower productivity than the average will have its performance adjusted upward.

Although individual plot adjustments are not possible, the lattice designs permit the performance of a genotype to be adjusted upward or downward according to the productivity of the blocks in which it was grown. The randomized complete-block design does not divide the replication into smaller units and is not able to adjust the performance of a genotype for environmental variation within replications.

The effectiveness of the lattice design in accounting for environmental variation within replications depends on the pattern of variation. Figure 19-9 shows two replications with variation in soil productivity. The soil productivity in

Block						
	Replication 1					
1	1	2	3	4	5	6
2	7	8	9	10	11	12
3	13	14	15	16	17	18
4	19	20	21	22	23	24
5	25	26	27	28	29	30
6	31	32	33	34	35	36
7	37	38	39	40	41	42
	Replication 2					
1	7	13	19	25	31	37
2	1	14	20	26	32	38
3	2	8	21	27	33	39
4	3	9	15	28	34	40
5	4	10	16	22	35	41
6	5	11	17	23	29	42
7	6	12	18	24	30	36
	Replication 3					
1	12	17	22	28	33	38
2	2	13	24	29	35	40
3	4	9	20	25	36	42
4	6	11	16	27	32	37
5	1	7	18	23	34	39
6	3	8	14	19	30	41
7	5	10	15	21	26	31

Figure 19-8 Lattice design for an experiment with 42 entries and three replications. (Adapted from Cochran and Cox, 1957.) For a randomized complete-block design, there are no blocks within a replication and the entries are assigned at random to the 42 plots.

replication 1 increases from left to right. The blocks of the lattice design are arranged in a pattern that effectively measures the variation, as evidenced by differences in the mean for each block. The variation in soil productivity in replication 2 does not fit a consistent pattern. Much of the variation occurs within blocks, and the mean performance of the blocks is relatively similar. The lattice

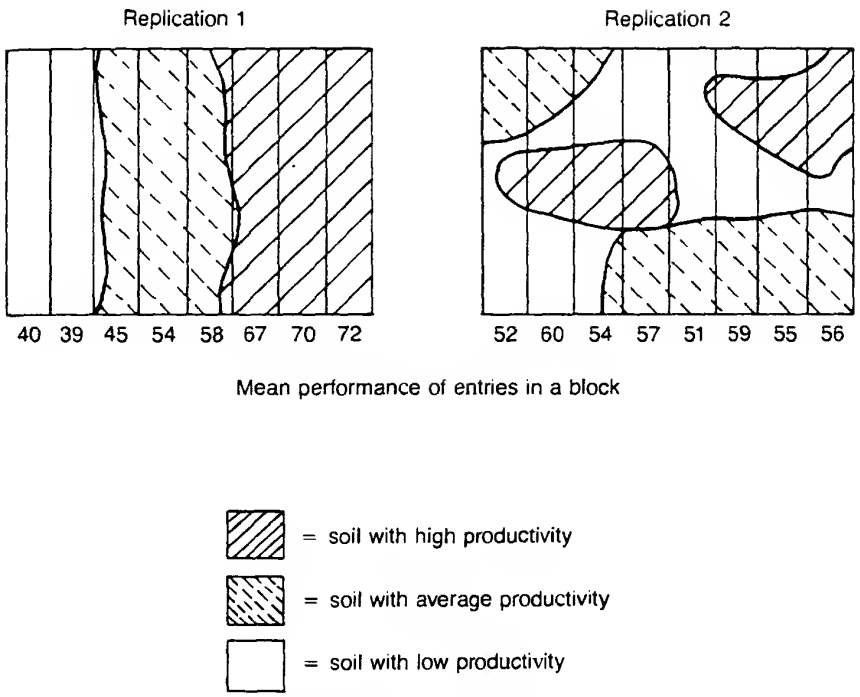


Figure 19-9 The effect of the pattern of variation in soil productivity on the effectiveness of the lattice design in accounting for environmental variation within a replication. The lattice would be more effective in replication 1 than in replication 2.

design cannot adjust for differences in productivity within a block; therefore, it would not be as effective in replication 2 as in replication 1.

The effectiveness of the lattice design compared with the randomized complete-block is expressed as relative efficiency. Relative efficiency is computed as a ratio of mean squares for experimental error of the two types of design.

$$\text{Relative efficiency} = \frac{\text{mean square for error of lattice}}{\text{mean square for error of randomized complete-block}} \times 100$$

The ratio is used to determine the number of replications that would have to be used with the randomized complete block to achieve a precision in detecting differences among the means of genotypes equal to that with a lattice design. A relative efficiency of 150 percent indicates that 50 percent more replication would have been needed with a randomized complete-block design than with a lattice.

The two types of design differ in the flexibility that is possible in a test. The randomized complete-block can accommodate any number of genotypes or replications. The lattice design requires that a specified number of genotypes and replications be included. For example, no lattice design can be used with 44, 58, or 74 genotypes. There is no restriction in a randomized complete-block for the length and width of a replication. For example, a test with 72 entries could be planted 8 plots long by 9 plots wide or 6 plots long by 12 plots wide. The shape of replication for a particular number of genotypes in a lattice is not as flexible. A test with 72 entries could be planted 8 plots long by 9 plots wide, not 6 plots long by 12 plots wide.

The randomization of an experiment and statistical analysis of data are more complex for a lattice than for a randomized complete-block. This can be important if the work is done by hand, but not if done by computer. Computer programs are available that will readily accommodate either type of design.

EQUIPMENT FOR EFFICIENT EVALUATION OF GENOTYPES

The efficient evaluation of a large number of genotypes is important for genetic improvement. Plant breeders have been actively involved in the development of equipment that permits them to evaluate more genotypes with equal or greater quality than was previously possible. The equipment ranges from simple hand devices to sophisticated computers.

Each crop has unique characteristics that influence the type of equipment used. Even for a certain crop, breeders differ as to the type of equipment they consider most desirable. Here only a small sample of available equipment will be used to illustrate how large numbers of genotypes are evaluated by plant breeders.

Preparation of Seed for Planting

The main steps involved in preparing a field experiment include packaging the seed and placing it in the proper arrangement for planting. Computers can be used to randomize entries and assign plot numbers. The computer system can print an adhesive label for each packet of seed to be packaged. The label contains the plot number, the entry number, and other information of value to the breeder. The plot and entry information also can be printed on pages used to record data in the field. The same work can be done by hand, but would require a large amount of labor and would be more subject to human error.

Seed is counted by hand or by electronic counting devices. If the number of seeds for a plot is large and precise numbers are not required, the seeds may be measured by volume.

Planting

Rapid planting of plots can be accomplished with engine-driven planters. Multiple-row plots may be planted from a single packet when each row does not require the exact same number of seeds. The seed is passed through a divider that separates the seed into a fraction for each row. The divider may be a powered spinning device or a gravity system.

The planter can move through the field without stopping. Seed for a row is placed in a container above a planting cone. When the row is to be planted, the container is lifted and the seed drops onto the planting cone. Two types of cones are used to distribute seed along the row. For one type, the base turns and carries the seed to the outlet. There it is knocked from the base by a stationary plate, falling through the outlet to the soil. This type of cone is used for relatively small seeds that do not roll easily, such as barley. The second type has fins mounted on the center cone. The seed falls onto a stationary base and is dragged by the fins to the outlet. The fins are well suited to relatively large seeds, particularly those that have a tendency to roll easily, such as maize and soybean. The length of a plot is a function of the distance traveled by the planter before all the seed has left the cone. At a constant ground speed, a cone must turn faster for short rows than for long rows. Adjustment of the speed of the cone rotation can be accomplished readily by several mechanical systems.

While the seed for one plot is being planted, the seed for the next plot is put in the container above the cone. There are a number of ways to determine when the container should be lifted to begin a plot. One way is to mark the beginning and end of each plot in the field before planting starts. When the planter reaches the beginning of a plot, the operator lifts the containers manually or electronically. The advantage of this procedure is that the location of each plot can be identified as soon as planting is complete. The second way is to use a cable extended across the field that has knobs spaced along it. The spacing between knobs is equal to the length of the plot and the alley. For plots that have rows 5 m long with a 1 m alley between them, the knobs would be spaced 6 m apart. As the planter passes by the cable, the knobs signal when the container should be lifted manually, or it activates an electronic tripping device. The cable is moved after each pass across the field. Use of the cable saves time at planting by eliminating the need to mark the start and end of plots manually.

Weed Control

Weed control is accomplished by the use of chemicals, cultivation, and hand weeding. The chemicals generally are those applied for weed control in commercial production of the crop. Cultivation equipment may be especially designed for use in research fields or may be the same equipment used commercially.

Preparation of Plots for Harvest

Trimming of plots to a constant length before harvest is done manually or with specialized equipment. Plots of small grains generally are trimmed to a constant length early in the season when the plants are about 30 cm tall. A rototiller or mower is passed along the end of each plot to kill the unwanted plants. The rototiller may be mounted on a tractor or may be a self-propelled unit that a person walks behind. Plots of soybean can be cut to a constant length with rotary mowers before seed filling begins. Two mowers are attached to a pipe so that they are separated by a distance equal to the desired plot length, and are driven perpendicular to the length of the rows.

Harvest

The most common type of harvester for the measurement of forage yield in the United States is a self-propelled flail chopper. The machine cuts the plants with a rotating flail that throws the cut portion into a collection point behind the driver. The plant material for a plot may be collected in a plastic container and weighed on a stationary scale set up in the field. To eliminate the labor required to use containers, an electronic scale can be mounted on the machine. The plant material is weighed and then it is discarded into a wagon.

The harvest of plots for their seeds is conducted with three different procedures or types of equipment. One procedure is to collect that part of the plant that bears the seed, weigh it directly, or carry it to a stationary machine for threshing. The plant part may be removed by hand or may be collected with a machine, such as a mower with a collection basket mounted behind the sickle. The harvested sample may be threshed immediately or dried for a period of time before threshing. One popular type of stationary machine is the Vogel thresher. The plants pass vertically through the machine as they are threshed. For a second type of stationary thresher, the material passes through the threshing cylinder and falls on a sieve that helps separate the seed from the plant debris. Air is used to separate the seed and the plant debris in both types of machine.

The second procedure for harvesting plots is to use a self-propelled thresher specifically designed for small plots. The plant part with the seed is gathered into the machine and passes through a threshing cylinder, then the seed and plant debris are separated by sieves and air. The seed may be placed into a bag and saved or may be weighed immediately and discarded. Seed harvested from self-propelled machines generally is more subject to mixtures than that harvested with a stationary thresher.

The third type of equipment is a commercial combine modified for the harvest of small plots. A commercial unit is used only when the amount of seed harvested

from a plot is relatively large and is not saved for planting. Modifications of the commercial combine include reduction of the number of rows harvested and the addition of equipment for weighing the seed.

Data Collection

Usually a number of characters are measured on each plot, such as height, standability, and yield. The data may be recorded in a field book, then manually entered into the computer for statistical analysis. Alternatively, the information may be recorded in an electronic data collector and transferred directly to the computer. This saves time and reduces the possibility of human error. Plot and entry designations also can be recorded on labels that can be read into the data collector by an electronic scanner.

Data Analysis

Computers facilitate the selection of lines by summarizing data in whatever manner is beneficial to the breeder. They save an extensive amount of time, minimize human error, and permit data to be summarized in a short period of time.

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